

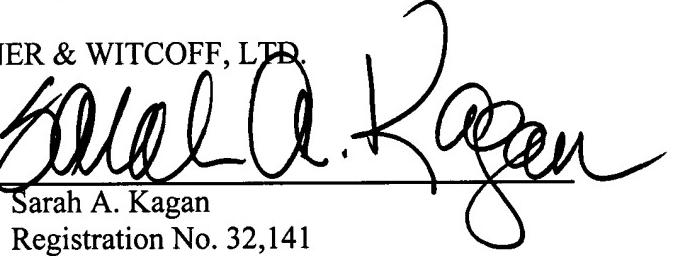
REMARKS

Claims 1-32, 34-47, 39-40, and 44-129 are presented for examination. Applicants respectfully solicit favorable consideration and allowance of the instant application.

Respectfully submitted,

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MARKED UP AMENDMENTS

IN THE SPECIFICATION:

Paragraph beginning at page 1, lines 4-11 has been amended as follows:

~~This application claims the priority of the U.S. Provisional Application, Serial No. 60035327, attorney docket No. 3004, filed on Jan. 13, 1997. This application is related to U.S. Application Ser. No. 08/529,115 filed on September 15, 1995, U.S. Application Ser. No. 08/168,904 filed December 15, 1993, which is a division of U.S. Application Ser. No. 07/624,114 filed December 6, 1990. U.S. Application Ser. No. 07/624,114 is a CIP of U.S. Application Ser. No. 07/362,901 filed June 7, 1990.~~
This application is a divisional of U.S. Application Ser. No. 09/086,285, filed May 29, 1998, which is a continuation-in-part of PCT application US98/0126 filed January 12, 1998, which claims priority to provisional application 60/035,327 filed January 13, 1997 (expired). All of the above applications are incorporated herein by reference.

Paragraph beginning at lines 3-21 of page 7 has been amended as follows:

Figures 98A and 98B illustrate fluorescence images of oligonucleotide arrays monitoring 1,650 genes in parallel (1 of a set of 4 arrays covering 6,600 genes). In Fig. 98A representative hybridization patterns of fluorescently labeled cRNA from normal (HT-125) and malignant breast (BT-474) cells are shown. The images were obtained after hybridization of arrays with fragmented, biotin labeled cRNA and subsequent staining with a phycoerytherin-streptavidin conjugate. Bright rows indicate messages present at high levels. Low level messages (1-10 copies/cell) are unambiguously detected based on quantitative analysis of PM/MM intensity patterns. In the lower portion of the Figure a magnified view of a portion of the array highlighting examples of altered gene expression between BT-474 and HT-125 is shown. In area 1, induced (>10-fold change in hybridization intensity) genes are shown, in area 2,

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unchanged (<2-folded change in hybridization intensity) are shown, and in area 3 repressed (>10-fold change in hybridization intensity) are shown. Fig. 9B8B illustrates zoom-in images of genes 1, 2, & 3 in (A) as 20 probe pairs of perfect-matched (PM) and single base mis-matched (MM) oligonucleotide probe cells. Average fluorescence intensity difference for PM-MM in HT-125 versus BT-474 (normalized to β -Actin and GAPDH signals) are shown in the three rows. In row 1 (Her2/neu oncogene) the average intensities are 111 versus 5,127; in row 2 (laminin receptor) 3,495 versus 6,088; and in row 3 (galectin-1) 7,952 versus undetected.

Paragraph beginning at lines 22-26 of page 7 has been amended as follows:

Figure 409 illustrates expression profiles of subset of genes from normal versus malignant breast cells. Average perfect match-mismatch (PM-MM) intensity differences (normalized to β -Actin and GAPDH signals) were plotted for the genes highlighted in Figure 98A that demonstrated greater than a 2-fold difference in hybridization signals between HT-125 and BT-474. Values for signals off scale are indicated.

Paragraph beginning at lines 27-32 of page 7 has been amended as follows:

Figure 410 illustrates p53 sequence analysis and mutation detection by hybridization. In Figure 410A, an image of the p53 genotyping array hybridized to 1,490 bp of the BT-474 breast carcinoma p53 gene (left) is shown. A zoom-in view of hybridization patterns of p53 wild-type reference and BT-474 DNA in a region of a G→A single-base mutation in BT-474 is shown at the right. In each column are 4 identical probes with an A, C, G or T substituted at a central position. The hybridized target sequence identified based.

Paragraph beginning at page 8, lines 1-12 has been amended as follows:

T substituted at a central position. The hybridized target sequence identified based on mismatch detection from left to right as the complement of the substitution base with the brightest signal. The G→A transition seen in BT-474 is accompanied by a loss of signal at flanking positions as these probes now have a single-base mismatch to the target distinct from the query position. Fig. 4*B10B* (top), comparison of wild-type reference (black) and BT-474 p53 gene (red) hybridization intensity patterns from sense (above) and anti-sense strands (below) in the region containing a mutation. The area shown demonstrates the “footprint” and detection of a single-base difference between the samples (vertical green line). GeneChip data analysis output is shown (bottom) that unambiguously identifies a G→A base change at nucleotide 1,279 of p53 in BT-474 resulting in a glutamic acid to lysine amino acid change in exon 8 (DNA binding domain). The upper portion of output displays the p53 wild-type reference sequence. Aligned outputs of wild-type p53 control and BT-474 samples are shown.

Paragraph beginning at page 52, lines 10-20 has been amended as follows:

Figure 9*A8A* (top panel) shows representative hybridization patterns of total message from normal and malignant breast cells to sets of 20 probe pairs from 1,650 gene sequences (one array of a set of 4 encompassing 6,600 human genes). Clear examples of unchanged and altered patterns of gene expression can be observed by visual comparison of the fluorescence intensities of probes sets from these two samples. The quantitative analysis of hybridization patterns is based on the assumption that for a specific mRNA the perfect-matched (PM) probes will hybridize more strongly on average than their mis-matched (MM) partners (Fig. 9*B8B*). The average difference in intensity between PM and MM hybridization signals is computed together with the average of the logarithm of the PM/MM ratios

for each probe set. These values are then used to determine the relative copy number of a detected message.

Paragraph beginning at page 53, lines 1-4 has been amended as follows:

hybridization signal intensities, that range over 4-orders of magnitude, revealed all categories of message expression changes including repressed (>10-fold down), down-regulated (<10-fold down), up-regulated (<10-fold up) and induced (>10-fold up) mRNAs between normal and malignant cells as shown in Fig. 409.

Paragraph beginning at page 53, lines 5-13 has been amended as follows:

Genes that are repressed and induced/activated may provide a particularly good starting point to decipher the molecular pathways involved in programs of tumorigenesis. To identify the genes falling into each of these two categories we sorted the normal and malignant message populations to identify those genes that demonstrated a 10-fold or greater difference in messages intensities. This analysis revealed 168 genes repressed and 137 genes activated in BT-474 when compared to HT-125, as shown in Table 3 (Figure 4410). 260 of the messages displaying differential expression corresponded to GenBank human full length genes, 45 to ESTs with homologies to other eukaryotic or viral genes.

IN THE CLAIMS:

Claim 11 has been amended as follows:

11. (Amended) A method for detecting a functional mutation in a target up-stream regulatory gene comprising:

preparing a reference sample from reference cells having a wild-type up-stream regulatory gene

corresponding to said target up-stream regulatory gene;

preparing a target sample from target cells suspected of having a mutation in said target up-stream regulatory gene, said target cells being otherwise substantially similar to said reference cells;

detecting the expression of a plurality of more than 5 down-stream genes in said reference sample to obtain a reference expression pattern, said down-stream genes being up or down regulated by said wild-type up-stream regulatory gene;

detecting the expression of said plurality of down-stream genes in said target sample to obtain a target expression pattern; and

comparing said reference expression pattern with said target expression pattern to detect functional mutation in or inactivation of said target gene.

Claim 16 has been amended as follows:

16. (Amended) The method of claim 11 further comprising the step of:

indicating a loss of wild-type function in said target gene if a significant number of said down regulated genes are expressed ~~relatively~~ higher in said target sample than in said reference sample or if a significant number of said up-regulated genes are expressed ~~relatively~~ lower in said target sample than in said reference sample.

Claim 17 has been amended as follows:

17. (Amended) The method of claim 11 further comprising the step of:

indicating a gain of function mutation in said target gene if a significant portion of said down regulated genes are expressed ~~relatively~~ lower in said target sample than in said reference sample or if a

significant portion of said up-regulated genes are expressed relatively higher in said target sample than in said reference sample.

Claim 29 has been amended as follows:

29. (Amended) A method for detecting a p53 gene functional mutation in target cells comprising the steps of:

preparing a reference sample from reference cells having a wild-type p53 gene, said reference cells being otherwise substantially similar to said target cells;

detecting the expression of a plurality of more than five down-stream genes in said reference cells and said target cells to obtain a target expression pattern and a reference expression pattern, said down-stream genes being up- or down-regulated by said wild-type p53 gene; and

comparing said reference expression pattern with said target expression pattern to detect said p53 functional mutation.

Claim 34 has been amended as follows:

34. (Amended) An in-cell functional assay for a p53 sequence alteration comprising the steps of:

preparing a target sample from target cells having said p53 sequence alteration;

preparing a reference sample from reference cells having a wild-type p53 gene, said reference cells being otherwise substantially similar to said target cells;

detecting the expression of a plurality of more than 5 down-stream genes in said reference cells to obtain a reference expression pattern and in said target cells to obtain a target

expression pattern, said down-stream genes being ~~up- or down regulated by said wild type p53 gene;~~
selected from the group consisting of p53 up-regulated genes and p53 down-regulated genes; and
comparing said reference expression pattern with said target expression pattern to
determine the function of said p53 sequence alteration.

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